CXLIII. THE POLYURONIDE CONSTITUENTS OF FORAGE GRASSES.

By HAROLD WILLIAM BUSTON.

*From the Biochemical Department, Imperial College of Science
and Technology, South Kensington.*

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DURING the past few years, an extended study has been made in these laboratories of the chemistry of certain constituents of the forage grasses, the nitrogenous substances and the fats and waxes having received particular attention. Samples of a number of grasses of pure strain and known history being available, the opportunity was taken to examine the cell-wall substances of certain of these. Detailed information on the chemistry of these substances is extremely scanty, being limited to such rather empirical data as "crude fibre," "pentosan" and cellulose content of the tissues; in view of the importance of the cell-wall materials from the standpoint of nutritional value and digestibility of the crops, a much more detailed study seemed to be called for. The polyuronides (pectins and hemicelluloses) of cocksfoot grass have been examined in some detail, while a number of analyses of the chief cell-wall and other carbohydrate constituents of other grasses, grown under comparable conditions, are recorded.

EXPERIMENTAL.

Material used.

The cocksfoot used in the large-scale preparations of pectin and hemicellulose was obtained in the first place from the Agricultural Research Station of Imperial Chemical Industries Ltd., at Jealott's Hill. The crop was taken 3 weeks after the plot had been closely cut and dressed with nitrogenous fertiliser; the grass at this time was from 8 to 10 in. high, and the upper 6 in. of the blades were removed with a scythe. The first sample (1930) was minced, pressed and the residue extracted with ether and boiling alcohol, as described by Pollard *et al.* [1931]; this material was used only for preliminary experiments. The later batches (1931) were oven-dried, ground and extracted with ether only, so that the whole of the pectin, *etc.* remained in the tissue.

The material of the 1933 series was grown at the Imperial College Biological Field Station, Slough; it was allowed to grow to about 1 ft., cut low with a scythe and dried rapidly in a large steam-oven; the material, after grinding, was used without ether extraction. The small samples of various grasses used for the comparative analyses were also grown at Slough; their history is detailed below (p. 1034).

In all cases, in both large- and small-scale work, the ground dried grass was passed through sieves, and the portion of 60–90 mesh used for the extractions, experiments having shown that this was the most suitable size of particle.

Pectic substances.

250 g. of dried cocksfoot (1930) were extracted with 0.5 % ammonium oxalate on a boiling water-bath for 20 hours. The filtrate was precipitated with excess of alcohol, the gelatinous precipitate was redissolved in about 500 ml. of warm water and again precipitated with alcohol containing a little hydrochloric acid. After thorough washing with graded strengths of alcohol and drying, the precipitate was obtained as a nearly white powder (about 4 g.), containing 1.5 % of ash. Determination of the true pectin content of the substance by the calcium pectate method gave a calcium pectate value of 81 %. A sample of the calcium pectate prepared from the material gave furfuraldehyde 19.50 %, uronic anhydride ($\text{CO}_2 \times 4$) 67.10 % (average), agreeing with the values accepted for pure calcium pectate. The grass pectin was therefore considered to be of normal type.

Estimations of the amounts of the individual pectic substances present in certain of the samples were made, following the methods outlined by Branfoot [1929]. It was found necessary to vary the procedure of Branfoot in one respect. Her method includes extraction of the pectin present in the cell-wall as protopectin by means of boiling *N*/75 hydrochloric acid and estimation of the pectin in this solution as calcium pectate directly, *i.e.* without the preliminary precipitation with acidified alcohol. Unless this precipitation was carried out, the values found for "protopectin" were considerably higher than those for "total pectin" (ammonium oxalate extract), *i.e.* the *N*/75 hydrochloric acid removed from the tissue some substance capable of giving an insoluble calcium salt under the conditions of the usual calcium pectate precipitation (see below, p. 1030). It may be pointed out that this difficulty would not have arisen with the alternative methods of extraction proposed by Nanji and Norman [1928].

The results of some of these estimations are given in Table I.

Table I. *Distribution of pectic substances.*

Results expressed as g. of calcium pectate per 100 g. dry grass;
mean of 3 or more values; see also Table V.

1	2	3	4	5	6
Sample no.	Total pectin (amm. oxalate extr.)	Pectin in HCl extr., (after alc. pptn.)	Pectin of middle lamella <i>etc.</i> (2-3)	"Pectin" in HCl extr., (omitting alc. pptn.)	Substance other than pectin in (5)
1930, Q	2.80	—	—	3.56	0.76
1931, 3	1.74	0.31	1.43	3.04	2.73
1931, 4	1.76	0.28	1.48	1.03	0.75

Methoxyl determinations were made on samples of pectin prepared from certain of the ammonium oxalate extracts by precipitation with acid alcohol and purified by reprecipitation, *etc.* The methoxyl values obtained were all below 3 %, after allowing for the non-pectic substances present as impurities in the material, on the basis of the calcium pectate values of the solids [*cf.* Norman, 1928]. It may be seen, however, from Table I, that by far the greater part of the pectin was extracted only by ammonium oxalate, *i.e.* occurred in the tissue as "middle lamella pectin" [Branfoot, 1929], which is usually regarded as being in the de-esterified or pectate form.

In order to obtain some further idea as to the degree of esterification of the pectin present otherwise than in the form of pectate, the following method was adopted. Ammonium oxalate extracts of the dried grass were made, the period

of extraction usually not exceeding 4 hours, at 98°; the extracts were precipitated with neutral alcohol, so that the insoluble pectic acid was not formed. The precipitate (containing ammonium pectate and soluble pectin) was well washed with 70 % alcohol and redissolved in water. In an aliquot portion of the solution, total pectin was determined in the usual manner. A further aliquot portion was treated directly with 0.4 *N* acetic acid and calcium chloride, without previous de-esterification with alkali; thus only the pectin present in the extract in the form of soluble pectate was precipitated as the calcium salt. In this manner it was possible to obtain an approximate estimate of the relative proportions of esterified and non-esterified pectin in the ammonium oxalate extracts; the assumption is made that no esterified pectin is precipitated by calcium chloride in the presence of acetic acid.

The results of these determinations, summarised in Table II, indicated that the major part of the pectic substances was indeed present in the de-esterified form. A recalculation of the methoxyl values of the esterified fractions of the various pectins, assuming that methoxyl groups were entirely absent from the "pectate-pectin" fractions, showed that (except in a single case) the average methoxyl content of the esterified pectin was still far below that required for the fully methylated derivative (11.76 % OMe). In three of the cases recorded, the calculated value was close to that required by the dimethoxyl ester, but this probably represented only a chance average figure. The unusually low methoxyl value found for sample 1930, Q, was probably due to the fact that in this case the ammonium oxalate extraction was allowed to continue for 20 hours; Norris and Schryver [1925] have shown that ammonium oxalate may produce considerable de-esterification of pectin during such prolonged periods of extraction.

Table II. *Methoxyl content of pectins.*

1	2	3	4	5
Sample	Ester-pectin as % of total pectin	Pectate-pectin as % of total pectin	Average OMe content of total pectin	OMe con- tent of ester- pectin, %
1930, Q	40	60	0.6	1.5
1931, 3	34.5	65.5	1.93	5.59
1931, 4	21	79	1.18	5.62
1932	30	70	1.90	6.33
1933, G	24	76	2.80	11.50

It has been pointed out above (Table I) that precipitation of the extract given by *N*/75 HCl, without preliminary precipitation by acid alcohol, gave very high values for "calcium pectate," suggesting the presence of some substance giving an insoluble calcium salt under the conditions of the calcium pectate precipitation. A portion of the grass was allowed to stand at room temperature with *N*/75 HCl for 4 weeks, the acid being changed five times during the period; the acid extracts gave no trace of an insoluble calcium salt when tested. It seemed therefore that the original contaminant was not an acid liberated from an insoluble salt by the action of the *N*/75 HCl (*e.g.* oxalic acid from calcium oxalate), but that mild hydrolysis was definitely necessary for its production. Apart from this observation, the nature of the substance has not been investigated. The need, in dealing with leaf extracts at any rate, of performing a preliminary alcohol precipitation on the *N*/75 HCl extracts, as well as on those of ammonium oxalate, was evident from these results; it is not indicated by Branfoot [1929].

Hemicelluloses.

In comparison with the chemistry of the pectins, that of the hemicelluloses remains obscure; they comprise a group of substances without any very distinctive common property, and the most usual definition classes them simply as carbohydrate substances, not removed from plant tissues by water, but extracted by 4 or 5 % alkali. Many of the isolated hemicelluloses themselves are however water-soluble.

For the preparation of fairly large amounts of the hemicelluloses, the procedure of Norris and Preece [1930] was followed, and the fractions obtained have been designated according to their nomenclature. Batches of 250 or 500 g. of the dried cocksfoot were extracted first with cold water for 24 hours, then with 0.5 % ammonium oxalate for 6 hours at 98°. After washing with hot water, the well pressed residues were allowed to stand overnight with cold 50 % alcohol, containing 1 % NaOH, allowance being made for water retained in the residues [*cf.* Preece, 1931]. The alcoholic liquor was pressed out and the residue allowed to stand for several days with successive portions of 4 % sodium hydroxide solution at room temperature. The combined extracts were acidified with acetic acid, whereby the crude hemicellulose A was precipitated as a thick green flocculum, whose colloidal nature rendered its removal a matter of extreme difficulty, even in the presence of considerable excess of acetic acid. The most satisfactory separation was obtained by filtering several times on paper-pulp, on the surface of which was spread a thin layer of animal charcoal; a clear filtrate was thus obtained, while the hemicellulose A was recovered by extracting the pulp with alkali and reprecipitating with acetic acid. Considerable loss was however unavoidable at this stage. The hemicellulose was dried by means of alcohol, whereby much colouring matter was removed; the final product was still brown in colour and was found to contain 3 % of ash and 2.5 % N (Kjeldahl), equivalent to approximately 15 % of protein. Further purification was carried out by conversion into the copper compound, according to the method of Baker and Pope [1900], and products were obtained with 0.18 to 0.35 % ash and from 0.4 to 0.5 % N; further purification by this method seemed impossible.

After removal of hemicellulose A, the clear filtrates were treated with half their volume of acetone, to precipitate fraction B, and finally with excess of acetone, to precipitate fraction C; the crude fractions were in most cases converted directly into their copper compounds, and thus further subdivided into fractions B 1, B 2 and C 1, C 2 [Norris and Preece, 1930]. These substances, when dry, were obtained as cream-coloured or white powders, possessing the usual characteristics of their class; with the exception of B 1, they were readily soluble in cold water, giving light yellow solutions. B 1 was soluble only in dilute alkali but was not then reprecipitated on neutralisation. The fractions were free from N but still contained amounts of ash up to 1 %. The total yield of isolated hemicelluloses amounted only to about 60 % of the amount present in the grass, as estimated on a small sample (see Table V). From 1100 g. of dried grass the yields of the individual fractions were: A, 33 g.; B 1, 18.8 g.; B 2, 4.5 g.; B (unfractionated), 17 g.; C 1, 8.7 g.; C 2, 13.2 g.; C (unfractionated), 9.5 g.; total, 104.7 g. The yield was expected to amount to about 200 g.; the greatest losses occurred (*a*) in the isolation of fraction A and (*b*) during the purification by means of the copper compounds.

Estimations of furfuraldehyde yield and carbon dioxide yield (on distillation with 12.5 % HCl) were made by the ordinary methods on each fraction.

Measurements of rotation were also made, in the case of fractions A and B 1, in 2 % sodium hydroxide solution, in the other cases in aqueous solution. Only in the case of one sample of hemicellulose A was it possible to determine the rotation, on account of the opacity of the alkaline solutions. The results, given in Table III, are the mean of two or more determinations in each case and are calculated on an ash-free basis.

Table III. *Hemicelluloses of cocksfoot.*

1	2	3	4	5
Fraction	Grass sample	Furfuraldehyde yield %	Uronic anhydride % (CO ₂ × 4)	Rotation, [α] _D
A	1931, 2	58.4	1.6	- 102° (c = 1.5 %, in 2 % NaOH)
B 1	1931, 3	53.1	10.6	- 47°
	1933, G	52.0	10.8	- 51° (c = 1.0 %, in 2 % NaOH)
B 2	1931, 3	16.4	3.4	- 53°
	1933, G	—	2.8	- 12° (c = 1.4 %)
C 1	1931, 3	54.0	6.5	- 83°
	1933, G	53.4	7.0	- 79° (c = 1.4 %)
C 2	1931, 3	45.4	10.0	- 66°
	1931, 4	—	—	- 69°
	1933, G	43.6	9.8	- 68° (c = 1.2 %)

In order to identify the sugar constituents of the hemicelluloses, portions of each were hydrolysed by boiling for 4 hours with 3.5 % sulphuric acid; excess of acid was removed by addition of the calculated amount of barium hydroxide solution. The filtrate in each case (distinctly acid except in the case of fraction A) was evaporated to small bulk, exactly neutralised with barium hydroxide and further evaporated to a syrup at low temperature; the syrup was extracted with warm 90 % alcohol. The alcoholic extract was evaporated again, the residue taken up in water, any barium present removed exactly and the liquid allowed to evaporate slowly *in vacuo*. In all cases except B 2, crystals readily formed in the syrups; these were removed and examined separately. The syrups were also examined for hexoses and pentoses by the usual methods. The qualitative examination of the five fractions for sugar constituents is summarised in Table IV.

Table IV. *Constituents of cocksfoot hemicelluloses.*

Fraction	Sugars, etc. present	Identified by	
A	{ Xylose	Rotation, [α] _D + 19°. Cd xylonobromide. Osazone	Xylose crystals readily
	{ Arabinose	Diphenylhydrazone, m.p. 201°	
B 1	{ Xylose	As above	Crystals pure xylose
	{ Arabinose	Mucic acid, by HNO ₃ , m.p. 218°	
	{ Galacturonic acid	Mucic acid, methylphenylhydrazone, m.p. 189°. Osazone (form of)	No crystals in syrup
B 2	{ Galactose	Diphenylhydrazone, m.p. 200°	
	{ Arabinose	As B 1, above	
C 1	{ Xylose	As B 1, above	Xylose crystals readily
	{ Arabinose	„	
	{ Uronic acid (?)	„	
	{ Galactose	Methylphenylhydrazone, m.p. 189°. Mucic acid, m.p. 218°	Xylose crystals slowly from syrup
C 2	{ Arabinose	Diphenylhydrazone, m.p. 201°	
	{ Xylose	Rotation, [α] _D = + 19.5°. Cd xylonobromide	
	{ Galacturonic acid	Mucic acid, m.p. 218° by Br oxidation	

The identification of the uronic acid constituents of the fraction was a matter of more difficulty; in no case did the uronic anhydride content exceed 11 %, and, as is well known, the free uronic acids are very readily decomposed by dilute acid during hydrolysis. In the case of fractions A and B 2, no attempt was made to identify the very small amounts of uronic acid present. In the other cases, use was made of the bromine oxidation method of Heidelberger and Goebel [1927], using both the original hemicelluloses and the barium salts obtained after hydrolysis; the nitric acid oxidation method was also used, except in the case of fraction C 2, which was found to contain galactose.

Hemicellulose A. This fraction was hydrolysed almost entirely to xylose; a trace of arabinose was detected, probably less than 2 % of the total sugars. The hydrolysis mixture, free from acid, had $[\alpha]_D = +20^\circ$; no hexose could be detected therein. Judging from the yield of furfuraldehyde given by the hemicellulose, since there was practically no uronic anhydride present, a small amount of hexosan was present. During the hydrolysis, an amount of dark-coloured, insoluble substance separated, equivalent to some 8–9 % of the hemicellulose. This contained 6 % of ash and gave 18 % of furfuraldehyde (on an ash-free basis).

Hemicellulose B 1. An estimation of the amount of arabinose in the hydrolysis mixture indicated the presence of about 15 % of this sugar; at most, only some 5 % of hexosan could have been present. A very small amount of mucic acid was obtained by oxidation with nitric acid; the small yield may have been due to the fact that the relatively large amount of sugar oxidation products prevented crystallisation; or another unidentified uronic acid may have been present.

Hemicellulose B 2. This fraction gave by far the lowest yield of furfuraldehyde; the hexosan content was calculated as 70 %. The total yield of the fraction was very small, and this, combined with the very low uronic anhydride content, made impossible any attempt to identify the acid constituent. It may be noted that, in this case only, the hemicelluloses prepared from different batches of grass showed markedly different rotations; this difference was further to be observed in the rotations of the hydrolysis mixtures. The 1931 preparation had $[\alpha]_D = +53^\circ$, while that of the 1933 batch hydrolysed in a similar manner was $+17^\circ$. The only sugars identified were galactose and arabinose; both of these are strongly dextrorotatory, so that the presence of some other sugar of low rotation was indicated. Mannose could not be detected. Judging from the yield of arabinosedi-phenylhydrazone given by the hydrolysis mixture some xylose was probably present, though none could be detected with certainty; the amount present however could not have accounted alone for the low rotations observed.

Hemicellulose C 1. The arabinose content of this fraction, estimated as in the case of fraction B 1, was found to be 15 %—approximately the same as in B 1. The only difference between the two fractions seemed to be in the slightly lower uronic anhydride content of this fraction. All attempts to identify the uronic acid in the hydrolysis mixture were unsuccessful.

Hemicellulose C 2. This fraction again contained much hexose, identified as galactose. Arabinose amounted to 24 % of the hydrolysate. These two sugars, with galacturonic acid, accounted for about 50 % of the hemicellulose, the remainder being xylose. Since galactose, arabinose and galacturonic acid are frequently found in association in polyuronides, the suggestion has been made that the pentose arises from the hexose by a series of mild oxidations, through the uronic acid; xylans, on the other hand, have been supposed to arise from

glucosans in a similar manner. With this in view, attempts were made to separate the xylan portion from this hemicellulose, since it seemed possible that it represented one item of a mixture, while the other three constituents were chemically combined. The methods attempted consisted in fractional precipitation, both from acid and alkaline solutions, but the furfuraldehyde, *etc.* yields of the fractions showed no significant variation from those of the original substance. On the other hand, the values obtained for the rotations of the fractions were all distinctly higher, values for $[\alpha]_D$ between -83° and -90° being recorded (aqueous solution, $c=0.7\%$), so that some intramolecular re-arrangement may have taken place.

"*Combined hemicellulose.*" Preece [1931] distinguishes between "free" and "combined" hemicelluloses, the latter being defined as that fraction extracted only by hot alkali. The hemicelluloses described above were all obtained by cold extraction; from one batch however the "combined" hemicelluloses were extracted by boiling the residues after extraction with cold 4 % sodium hydroxide for 3 hours. It was found that the extract contained only a trace of hemicellulose A, fractions B and C being present in roughly equal amounts. This was somewhat unexpected, since in the isolated state fraction A is the least soluble of the hemicelluloses.

Distribution of carbohydrate fractions in various grasses.

Small samples of various grasses, grown under comparable conditions on the experimental plots at Slough, were used for a series of estimations of certain of the cell-wall and other constituents. The plots, after cutting back closely in spring (1933), were dressed with ammonium sulphate. When the blades were some 3 inches high, the plots were again cut back closely and then allowed to grow on for a further week without receiving any further ammonium sulphate. At the end of a week, the growths were again cut down, and this crop was that used for the analyses described below. (Further details of the treatment given to the experimental plots are given by Chibnall *et al.* [1933]). The samples were all rapidly dried at $98-100^\circ$, ground and sieved, the 60-90 mesh portion being used throughout. In every case, the material was re-dried in a steam-oven for 24 hours before sampling.

The following estimations were performed on each of the grasses: ash, water-soluble substances; "reducing substances" before and after hydrolysis; pectin; hemicelluloses; cellulose; xylan associated with cellulose.

1. *Ash.* Determined on portions of 0.3 to 0.5 g. of dried grass.
2. *Water-soluble substances.* 2 g. samples of dried grass were allowed to stand for 24 hours in the cold with 100 ml. of water; the extract was removed, and the residue extracted twice with 100 ml. water for 4 hours, on the steam-bath. The combined extracts were evaporated to 100 ml. Total dry matter was estimated by drying aliquot portions in the steam-oven for 48 hours.
3. *Reducing substances.* Portions of the above aqueous extract were used for estimations of free sugar (*plus* other simple reducing substances), calculated as glucose, by Bertrand's method. Similar estimations were made on portions of the extract, following hydrolysis with 1 % sulphuric acid for 40 minutes. Longer hydrolysis resulted in loss of reducing power, due possibly to destruction of fructose.
4. *Pectic substances.* 3 g. of dry material were extracted twice with 100 ml. of 0.5 % ammonium oxalate for 6 hours on the steam-bath; the residue was well washed with boiling water. Pectin was estimated in aliquot portions of the extract by the usual calcium pectate method, after preliminary precipitation with acid alcohol.
5. *Hemicelluloses.* No satisfactory standard method is yet available for the determination of this group of substances in plant tissues. In the present case they were estimated by a method

Table V. *Cell-wall constituents of various grasses.*

All figures are mean of duplicates and are calculated as % of dried grass.

Species	Date of sampling	Period of growth days	Ash %	Reducing			Total pectin (as Ca pectate) %	Total hemi-cellulose A (ash- and N-free) %	Hemi-cellulose B (ash-free) %	Hemi-cellulose C (ash-free) %	C and B cellulose (including xylan) %	Xylan in C and B cellulose %	
				Total water-soluble material %	sub-stances (as glucose) %	sub-stances after hydrolysis %							
Rough-stalked meadow grass (<i>Poa trivialis</i>)	12. vi. 33	8	9.77	29.70	3.25	11.25	0.82	16.17	5.40	—	4.30	22.18	3.15
Timothy (<i>Phleum pratense</i>)	12. vi. 33	8	8.18	23.2	2.25	6.87	1.54	17.22	—	7.10	3.60	20.83	4.32
Chewing's fescue (<i>Festuca rubra</i> var. <i>fallax</i> Hack	9. vi. 33	8	8.32	23.0	1.01	4.70	0.90	20.70	2.76	13.55	4.37	21.38	3.89
Tall fescue (<i>Festuca elatior</i>)	9. vi. 33	8	8.55	25.5	1.87	5.62	1.62	19.92	2.77	11.02	5.70	22.56	1.86
Meadow fescue (<i>Festuca pratensis</i>)	9. vi. 33	8	7.93	24.9	1.25	6.25	1.60	18.47	3.95	9.16	5.41	24.07	3.68
" "	23. vi. 33	14	—	25.1	—	3.80	2.10	17.16	2.40	8.90	5.52	—	—
Italian ryegrass (<i>Lolium italicum</i>)	12. vi. 33	8	10.1	24.7	2.87	9.05	1.04	17.60	5.26	—	2.90	23.95	3.59
Crested dog's tail (<i>Cynosurus cristatus</i>)	12. vi. 33	8	9.08	24.5	3.67	13.10	1.02	16.30	—	—	3.40	23.25	3.92
Cocksfoot (<i>Dactylis glomerata</i>)	18. xi. 33	8	8.45	20.8	—	2.50	1.60	17.90	7.81	5.13	5.76	23.05	—
" "	27. vi. 33*	—	8.16	21.9	3.54	5.51	1.97	18.31	—	—	—	33.20	8.50
" "	1931*	—	6.46	19.3	3.94	5.47	1.76	18.55	—	—	—	31.64	—

* Not previously cut; growth 12 in. high.

similar to that of Preece [1931], involving actual isolation and weighing of the fractions. To obtain concordant results it was found necessary rigidly to standardise the procedure; this precaution being observed however the method seemed satisfactory.

The residues from the pectin estimations above were air-dried and then allowed to stand for 24 hours in 50 % alcohol containing 1 % NaOH, in order to remove "lignosaccharide" [Preece 1931]. The residues from this extraction were treated exhaustively with 4 % NaOH in portions of 50 ml.; two extractions were made at room temperature, for 48 hour periods, and five further extractions on a boiling water-bath for 6 hour periods; finally the residues were boiled for 1 hour under reflux with the alkali. The total extracts were made up to 400 ml. and centrifuged in order to remove a certain amount of fine suspended matter present. The following estimations were performed:

(a) *Total hemicellulose.* 50 ml. of the extract were treated with 10 ml. of glacial acetic acid, followed after 30 minutes by 270 ml. of 95 % alcohol; the mixture was allowed to stand for 48 hours, when the solid was collected on the centrifuge and allowed to stand for 48 hours in 50 ml. of 80 % alcohol. The centrifuging and washing were repeated, and the solid was finally filtered off on a tared alundum crucible, washed with absolute alcohol and dried in an oven at 98° overnight. Care was taken, during this filtration, that the solid collected in a small compact mass on the crucible; this being done, the small "button" of solid was readily detached when dry. The weight of solid was corrected for ash and protein content ($N \times 6.25$), these being estimated by the micro-methods.

(b) *Hemicellulose A.* 100 ml. of the extract were treated with 20 ml. of glacial acetic acid and allowed to stand for 3–4 days, and the precipitate was removed on the centrifuge. (In some cases, this was difficult; a fresh extraction, etc. was made in such cases.) The precipitate was washed with dilute acetic acid containing a little alcohol, then transferred to 50 ml. of 80 % alcohol, filtered and dried as before. The weight was again corrected for ash and protein.

(c) *Hemicellulose B.* After removal of hemicellulose A, the clear liquid (neglecting the washings) was treated with 70 ml. of acetone. After standing for 48 hours, the solid was removed, washed and dried as usual. A correction was applied for ash only.

(d) *Hemicellulose C.* The liquid remaining after removal of fractions A and B was precipitated with 400 ml. of 95 % alcohol, and the flocculum was removed after 48 hours, washed and dried as before. The weight was corrected for ash only.

6. *Cellulose* was estimated in portions of 2 g. by the Cross and Bevan method.

7. *Xylan in cellulose.* The furfuraldehyde yield of the Cross and Bevan cellulose was determined in each case and the xylan content of the cellulose calculated therefrom. (Hydrolysis of the Cross and Bevan cellulose from cocksfoot by the method of Monier-Williams had shown the presence of xylan in this product.) The figures given in Table V are calculated back to show the amount of this xylan in the original dry grass.

DISCUSSION.

The pectic substances of grass have been shown to be normal in type; the amount in the tissue is definitely low. With this low pectin content is associated a relatively high content of hemicelluloses, this being in accordance with the usual observations. The relative amounts of the two classes of substance are of the order met with in lignified tissues such as timber and straws, rather than in leaves. It has been considered generally that a high content of hemicellulose is associated with a prolonged period of development, or a slow rate of growth of the tissues; that such is not necessarily the case seems indicated by the observation that grass blades of only 8–10 days' growth contain some 20 % of hemicellulose. On the other hand, such figures as have been obtained seem to show that a distinct increase of cellulose and xylan associated therewith occurs with increasing age.

With regard to the individual polyuronides, the pectic substances have been shown to exist mainly in the form of pectates; the average methoxyl content of

the pectins is low. Five hemicellulose fractions have been distinguished, but how far these can be regarded as definite entities is doubtful. Two at least of the fractions (B 1 and C 1) were very similar chemically, as regards the proportions of the constituent sugars. In the case of cocksfoot, the fractions isolated from the 1931 and 1933 batches had practically the same constants except in the case of the very small fraction B 2 (Table III), so that one may conclude that the method of separation is capable of distinguishing fairly definite fractions in the case of any particular plant tissue. The variation in fraction B 2 is not explained.

Amongst the individual sugars isolated, xylose accounts for by far the greater part. Arabinose is also widely distributed amongst the various fractions, but galactose is confined to two of the hemicelluloses. No other sugar was detected among the hemicellulose hydrolysis products, but it may be remarked that a water-soluble polysaccharide, yielding fructose on hydrolysis, was found.

Of the uronic acids, only galacturonic acid was detected with certainty, in fractions B 1 and C 2; both these fractions gave also arabinose, and it is assumed that this sugar and the uronic acid have a common origin. Although xylose was present in such large amounts in all the hemicelluloses, the corresponding uronic acid (glycuronic acid) could not be detected.

SUMMARY.

1. The pectins and hemicelluloses of cocksfoot have been studied. Five types of the latter have been distinguished, yielding on hydrolysis mixtures of xylose, arabinose and galactose together with uronic acids. Xylose accounts for the greater part of the sugars in the hydrolysis mixture.

2. Comparative analyses of certain of the cell-wall constituents of a number of different grasses are recorded.

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